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Original Article

Aldolase C Profiling in Serum after Mild Traumatic Brain Injury: A Prospective Cohort Study

Kaveh Haddadi[1], MD; Siavash Moradi[2], MD; Leila Asadian[3], MD; Seyed Hosein Montazer[4], MD; Seyed Mohammad Hosseininejad[5], MD; Iraj Golikhatir[6], MD; Saeid Abedian Kenari[7], PhD; Abdulrassol Alaee[8], MD; Farzad Bozorgi[4], MD

[1]Department of Neurosurgery, School of Medicine, Orthopedic Research Center, Mazandaran University of Medical Sciences, Sari, Iran;
[2]Education Development Center, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran;
[3]Student Research Committee, Mazandaran University of Medical Sciences, Sari, Iran;
[4]Department of Emergency Medicine, School of Medicine, Orthopedic Research Center, Mazandaran University of Medical Sciences, Sari, Iran;
[5]Department of Emergency Medicine, School of Medicine, Diabetes Research Center, Mazandaran University of Medical Sciences, Sari, Iran;
[6]Department of Emergency Medicine, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran;
[7]Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran;
[8]Department of Radiology, School of Medicine, Mazandaran University of Medical Sciences, Sari, Iran

Correspondence:
Farzad Bozorgi, MD;
Department of Emergency, Imam Khomeini Hospital, Amir Mazandarani Ave., Postal code: 48166-33131, Sari, Iran
Tel/Fax: +98 11 33361630
Email: emergencmed@yahoo.com
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What's Known

- After a traumatic brain injury (TBI), in addition to clinical indices, the serum level of neurological biomarkers may provide valuable diagnostic and prognostic information.
- Current studies on neurological biomarkers have mainly focused on severe TBI. Serum aldolase C (ALDOC) level is shown to increase in less than an hour after TBI in mice.

What's New

- The diagnostic value of ALDOC for early detection of brain damage in patients with mild TBI (mTBI) is investigated.
- The median ALDOC serum level in the patients with positive CT scan findings was significantly higher than those with negative CT scan findings.

Abstract

Background: After a traumatic brain injury (TBI), in addition to clinical indices, the serum level of neurological biomarkers may provide valuable diagnostic and prognostic information. The present study aimed to investigate the aldolase C (ALDOC) profile in serum for early diagnosis of brain damage in patients with mild TBI (mTBI) presented to the Emergency Department (ED).

Methods: A single-center prospective cohort study was carried out in 2018-2019 at Imam Khomeini Hospital affiliated with Mazandaran University of Medical Sciences, Sari, Iran. A total of 89 patients with mTBI were enrolled in the study. Blood samples were taken within three hours after head trauma to measure ALDOC serum levels. Brain CT scan was used as the gold standard. Statistical analysis was performed using the Kruskal Wallis, Mann-Whitney U, and Chi square tests. The receiver-operating characteristic (ROC) curve plot was used to determine the optimal cutoff point for ALDOC. The sensitivity and specificity of the determined cutoff point were calculated. P values less than 0.05 were considered statistically significant.

Results: Of the 89 patients, the CT scan findings showed a positive TBI in 30 (33.7%) of the patients and in 59 (66.3%) a negative TBI. The median ALDOC serum level in the patients with positive CT scan findings (8.35 ng/mL [IQR: 1.65]) was significantly higher than those with negative CT scan findings (5.3 ng/mL [IQR: 6.9]) ($P<0.001$). The optimal cutoff point for ALDOC serum level was 6.95 ng/mL, and the area under the curve was 99.6% ($P<0.001$). The sensitivity and specificity of the determined cutoff point were 100% and 98%, respectively.

Conclusion: The ALDOC serum level in patients with mTBI significantly correlates with the pathologic findings of the brain CT scan. This biomarker, with 100% sensitivity, is a suitable tool to detect brain structural abnormalities in mTBI patients.

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Keywords ● Serum ● Brain injuries ● Traumatic ● Biomarker

Introduction

Traumatic brain injury (TBI) is caused by traumatic events that either damage the structure of the brain or disrupt its physiological function.[1] TBI occurs in 69 million (95% CI: 64-74 million) people worldwide annually.[2] Its severity is classified into mild, moderate, and severe categories based primarily on the patient's level of consciousness in the first 24 hours after head injury.[3] Mild TBI (mTBI) is the most common form of TBI that occurs in the age group 16-59 years with an overall incidence of 302 per 100,000

person-years (95% confidence interval 281-324). The incidence rate in the age group 16-20 years is 835 per 100,000 person-years in men and 726 in women.[4] TBI evaluation is made based on neurological imaging findings, such as a computed tomography scan (CT scan). CT scan has a low sensitivity for mTBI and exposes patients to significant radiation doses.[5]

Given that a million people are admitted annually to the emergency department (ED) and discharged without hospitalization, using imaging studies to rule out brain pathologies in patients with TBI increases unnecessary procedures and medical care for both the patient and the medical center.[5] Currently, researchers are investigating mTBI at cellular and molecular levels, since brain imaging findings can be used for diagnosis and peripheral blood samples for predicting brain damage. Identifying a marker for early diagnosis of brain injury is of particular interest.[6] In contrast with cardiac events, patients admitted to ED with mTBI do not demonstrate any immediately appreciable clinical symptoms, and the clinical use of blood biomarkers is therefore not common to facilitate rapid diagnosis and treatment of mTBI.[7] In recent years, however, neuro-biomarker studies have mainly focused on severe TBI. Since more than 80% of TBIs are mTBI, it is essential to find a fast and reliable biomarker test that will allow ED physicians to diagnose this group of patients.[8] Among several recent studies on glial cells and neuronal biomarkers, the most widely studied biomarkers are S100B, glial fibrillary acidic protein (GFAP), and ubiquitin C-terminal hydrolase-L1 (UCHL1).[9] However, these biomarkers were shown to have delayed appearance in serum after brain injury. Astrocytic biomarkers such as aldolase C (ALDOC), brain lipid-binding protein (BLBP), and phosphoprotein enriched in astrocytes 15 (PEA15) are new promising biomarkers that have been shown to appear in serum in less than one hour after brain injury.[10] ALDOC, unique in both astrocyte enrichment and abundance amongst proteins of the brain, is an astroglial injury-associated protein that can potentially be used to predict brain injury.[11] Brain injury causes astroglial protein depletion and disassembly in wounded and dying cell populations. According to several animal model studies, ALDOC is strongly associated with cell wounding and cell death.[11] The present study aimed to investigate ALDOC profiling in serum for the early diagnosis of brain damage in patients with mTBI presented to our ED.

Materials and Methods

A single-center prospective cohort study was conducted in 2018-2019 at Imam Khomeini Hospital affiliated with Mazandaran University of Medical Sciences, Sari, Iran. The study was approved by the Clinical Research Development Unit of the hospital as well as the Ethics Committee of the University (code: IR.MAZUMS.IMAMHOSPITAL.REC.1397.002). Written informed consent was obtained from all the participants or their parents/legal guardians in case of an acute medical condition.

A total of 89 patients with mTBI were enrolled in the study, and their data were analyzed. A brain CT scan is considered the gold standard for the diagnosis of mTBI. Inclusion criteria were TBI during the last 24 hours, a Glasgow coma scale (GSC) score of 13-15, and presentation of one or more of the following symptoms: post-traumatic loss of consciousness less than 30 minutes, amnesia immediately before and/or after trauma lasting less than 24 hours, temporary or permanent focal neurologic signs and symptoms, nausea, and vomiting. Exclusion criteria were age <18 years, pregnancy, spinal cord injury, and a history of psychosis, neurological disorders, and cancer.

A checklist was completed by the emergency medicine resident within the first three hours of a traumatic event. The list included information such as demographic characteristics of the patient, the cause of injury (vehicle crash, fall, heavy object), injury-associated symptoms (dizziness, nausea and vomiting, headache, tinnitus, pre-and/or post-traumatic amnesia), level of consciousness based on the GSC, and duration of the incident (in minutes). Upon admission, 10 cc venous blood samples were taken from the patients to examine biomarker levels. Bearing a trauma event in mind, a brain CT scan was immediately done. According to the clinical practice guidelines, a clinical neuro-radiologist blinded to the experiment performed all radiologic studies. The CT scan findings were also confirmed by an emergency medicine specialist and a neurosurgeon. Blood samples were transferred to the hospital laboratory in accordance with the blood cold chain system. After centrifugation and serum separation, ALDOC measurement was performed using an automated biochemistry analyzer (Stat Fax-2000, USA) and ALDOC ELISA kit (96T) (Zellbio, Germany). ALDOC assay was performed based on a double antibody sandwich method using the avidin-biotin system. The wells of the ELISA plate were percolated with an anti-ALDOC monoclonal antibody. The biotin-labeled ALDOC antibodies were mixed with streptavidin-HRP (assay range: 0.75-24 ng/mL, sensitivity: 0.02 ng/mL). The optical density (OD) values were used to

plot the standard curve using point-to-point calculation mode.

Statistical Analysis

Data were analyzed using IBM SPSS software, version 25.0. Data distribution was examined using the Kolmogorov-Smirnov test. Quantitative and qualitative data were expressed as median (interquartile range [IQR]) and frequency (percentage), respectively. The Kruskal-Wallis, Mann-Whitney U, and Chi squared tests (or Fisher's exact test) were used for statistical analysis. The receiver-operating characteristic (ROC) curve plot was used to determine the optimal cutoff point for ALDOC and its predictive accuracy for positive CT scan findings. A 2×2 table was drawn to calculate the sensitivity and specificity of the determined cutoff point. P values less than 0.05 were considered statistically significant.

Results

A total of 100 patients suffering from head trauma with the primary diagnosis of mTBI were enrolled in the study. Based on the exclusion criteria, nine patients were excluded from the study because of spinal cord injury (n=3), pregnancy (n=3), psychosis (n=1), and unavailability due to imprisonment (n=2). A further two patients refused to undergo the imaging study and were thus excluded. Of the remaining 89 patients, 30 (33.7%) had positive CT scan findings, and 59 (66.3%) had negative brain CT scan findings for TBI. Demographic characteristics of the patients and the cause of injury are shown in table 1. GCS scores were significantly lower in the mTBI patients with positive than negative brain CT scan findings (P<0.001). A comparison between clinical features with CT scan findings is shown in table 2. Nausea was the most common symptom of mTBI patients with positive CT scan findings (73.3%).

The results of the Kolmogorov-Smirnov test showed that none of the variables were normally distributed (P<0.05). Therefore, quantitative variables were described using median and interquartile range (IQR). The median ALDOC serum level in the patients with positive CT scan findings (8.35 ng/mL [IQR: 1.65]) was significantly higher than those with negative CT scan findings (5.3 ng/mL [IQR: 6.9]) (P<0.001). Moreover, among various categories, patients' sex had a statistically significant effect on median ALDOC serum concentration (P=0.031) (table 3). The diagnostic accuracy of ALDOC serum level was evaluated using the ROC curve plot. The optimal cutoff point for ALDOC serum level was 6.95 ng/mL (figure 1), and the area under the curve was 99.6% (P<0.001, 95% CI: 98.8-100); indicating high accuracy of the diagnostic test. The sensitivity and specificity of the determined cutoff point were 100% (95% CI: 88.43-100%) and 98.31% (95% CI: 90.91-99.96%), respectively.

Table 1: Presenting demographic characteristics and the cause of injury in patients with positive or negative computed tomography scan findings

Variable		Positive CT scan (n=30)	Negative CT scan (n=59)	P value
Age (years)	Median (IQR)	35.5 (32.5)	32 (15)	0.430*
Sex	Male (n, %)	26 (86.7)	42 (71.2)	0.080**
	Female (n, %)	4 (13.3)	17 (28.8)	
The cause of injury	Motor vehicle accident (n, %)	25 (83.3)	55 (93.2)	0.050**
	Fall (n, %)	5 (16.7)	2 (3.4)	
	Struck by heavy object (n, %)	0 (0)	2 (3.4)	
Time to admission (minute)	Median (IQR)	30 (15)	30 (10)	0.830*
GCS	Median (IQR)	14 (0)	15 (0)	<0.001*

*Mann-Whitney U test; **Chi squared test; P<0.05 is considered statistically significant; GCS: Glasgow coma scale; IQR: Interquartile range; CT: Computed tomography

Table 2: A comparison of clinical features in patients with positive or negative computed tomography scan findings

Symptoms	Positive CT scan (n=30)	Negative CT scan (n=59)	P value*
Dizziness (n, %)	5 (16.7)	14 (23.7)	0.991
Nausea (n, %)	22 (73.3)	38 (64.4)	0.791
Vomiting (n, %)	14 (46.7)	24 (40.7)	0.152
Headache (n, %)	14 (46.7)	28 (47.5)	0.990
Tinnitus (n, %)	0 (0)	0 (0)	-
Post-traumatic amnesia (n, %)	10 (33.3)	22 (37.3)	0.802
Pre-traumatic amnesia (n, %)	0 (0)	1 (1.7)	0.990
Post-traumatic seizures (n, %)	0 (0)	0 (0)	-

*Chi-squared test; P<0.05 is considered statistically significant.

Table 3: Presenting the aldolase C serum level for each of the studied categories

		Median ng/mL (IQR)	P value
Type of injury	Negative	5.3 (6.9)	0.781*
	Epidural hemorrhage	8.0 (2.7)	
	Subdural hemorrhage	8.3 (5.8)	
	Contusion	8.3 (2.3)	
	Intracranial hemorrhage	8.0 (0)	
	Subarachnoid hemorrhage	9.0 (1.0)	
Sex	Male	6.35 (2.75)	0.031**
	Female	5.10 (3.1)	
Age	<20	8.2 (3.9)	0.202*
	21-40	5.75 (2.7)	
	41-60	6.6 (4.8)	
	>60	6.5 (2.6)	
The cause of injury	Motor vehicle accident	5.9 (2.7)	0.050*
	Fall	7.4 (3.1)	
	Struck by heavy object	1.4 (0.8)	

*Kruskal-Wallis test; **Mann-Whitney U test; P<0.05 is considered statistically significant; IQR: Interquartile range

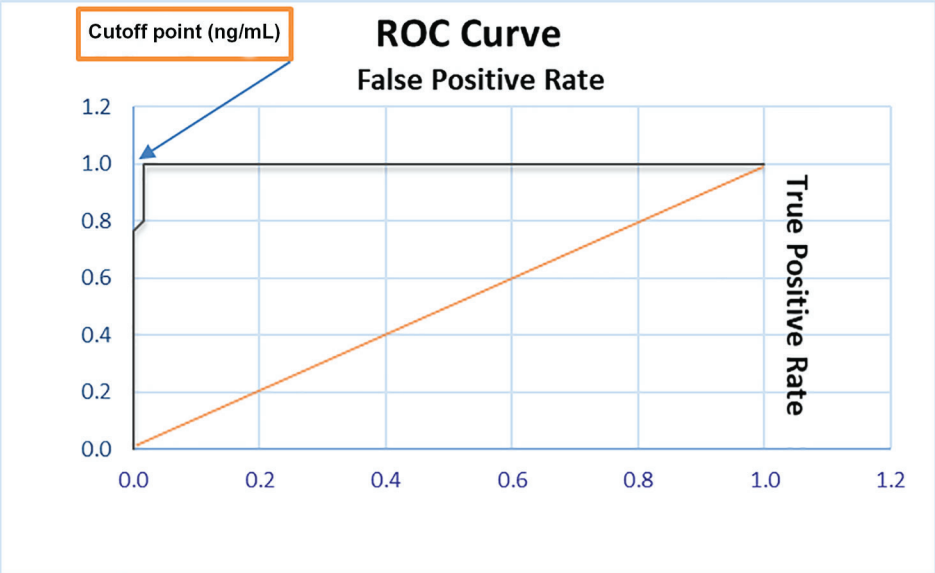

Figure 1: Determination of the optimal cutoff value for aldolase C using the receiver-operating characteristic (ROC) curve plot.

Discussion

The current practice of predicting the outcome of mTBI based on clinical and brain CT scan findings can be improved by determining ALDOC serum levels upon ED admission, particularly within the first three hours after a trauma event. A serum level >6.95 ng/mL is significantly associated with positive brain CT scan findings, including intracranial hemorrhage, with high sensitivity and specificity (100% and 98%, respectively). Among other variables, age and time to admission are not associated with positive brain CT scan findings. GCS scores, however, are significantly lower in mTBI patients with positive than negative brain CT scan findings.

Until recently, most studies investigating the role of neurological biomarkers in predicting the outcome of TBI have focused on severe TBI. This is while approximately 80% of all TBI cases admitted to the ED are of the mTBI category. Therefore, in addition to imaging studies to detect brain structural abnormalities in TBI patients, there is a growing need for a reliable and accessible biomarker that can help physicians with the diagnosis of patients, who need further evaluation. In recent years, several studies have proposed various markers for brain injuries such as neuronal cell body, astrocyte, delayed axonal injury, and demyelination. Neuronal cell body injury markers include neuron-specific enolase (NSE) and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1). Cerebrospinal fluid (CSF) and NSE levels in serum were reported to increase in severe TBI[12, 13] and mTBI.[14-16] UCH-L1 concentration also elevates in the plasma after mTBI, however, this marker is mostly used for the long-term prognosis of severe TBI.[17]

Although several studies have shown that UCH-L1 significantly increases after TBI and concussion,[18-20] its ability to predict brain structural abnormalities in TBI patients is still unclear due to the lack of evidence. Astroglial biomarkers, including S100B protein and GFAP, are also promising neurological biomarkers to guide TBI diagnosis. The S100B biomarker were shown to have a good sensitivity in predicting brain abnormality.[21-23] However, it can also be released from adipose tissue and cardiac/skeletal muscles, and its levels can be elevated due to orthopedic trauma without head injury.[24] Elevation of GFAP levels in CSF has been reported in the animal models of severe TBI[25, 26] as well as in the serum/plasma samples of mTBI models. Evidence suggests that the post-TBI elevation of GFAP is severity-dependent[26] and associated with CT scan pathological findings. However, more human studies are required to determine its sensitivity and specificity.[27-29] There is also another group of markers rising after TBI, namely delayed axonal injury and demyelination markers, including neurofilament protein (NF) and myelin basic protein (MBP).[30]

A previous study showed that ALDOC is persistently elevated up to five days after injury, while other widely used markers, such as GFAP and S100B, decreased on day two after injury.[31] In line with the findings of a controlled trial in rats,[11] ALDOC signals were reported to be present in the CSF and blood samples at three and 34 hours. Therefore, we recommend human studies to examine the use of such biomarkers in predicting brain injury in mTBI patients. Our results showed that ALDOC serum levels were significantly higher in the mTBI patients with positive than negative brain CT scan findings. In addition to optimal sensitivity and specificity, ALDOC has a negative predictive value of 100%, indicating its potential as a screening tool to avoid unnecessary imaging studies. The main limitation of our study was the absence of pediatric patients in the study population.

Conclusion

The initial assessment of TBI patients mainly relies on GCS scores and CT scan findings. Our findings indicate the applicability of ALDOC in predicting the need for a brain CT scan in mTBI patients, e.g., intracranial hemorrhage. ALDOC serum level above 6.95 ng/mL is shown to have a sensitivity and specificity of 100% and 98%, respectively, and a negative predictive value of 100%. Therefore, a brain CT scan can be advised for mTBI patients with ALDOC serum levels greater than 6.95 ng/mL within three hours after a trauma event. As a direct result, patients will not be unnecessarily exposed to radiation, thereby minimizing medical costs. Multicenter research studies are recommended to substantiate our findings.

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Conflict of Interest: None declared.

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